Charcot-Leyden Crystals

Formation from Primate and Lack of Formation from Nonprimate Eosinophils

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Charcot-Leyden crystals develop spontaneously in certain diseases of man and can be formed within minutes from eosinophils lysed with a surface-active agent (Aerosol-OT). Treated human eosinophils examined by electron microscopy showed the general features of cell lysis. After disruption of the eosinophilic granule of man, there remained an insoluble crystalline core, which was now more dispersed, a few tubular structures resembling microtubules and a fine granular accumulation around the periphery of the granule. It is suggested that such dispersion represents rearrangement of the crystalline structure allowing the material of the core to be incorporated into a Charcot-Leyden crystal. In guinea pig eosinophils, Charcot-Leyden crystals were not found even after prolonged lysis. By electron microscopy, the cellular changes in guinea pig eosinophils were generally similar to, but developed more slowly than, those in human eosinophils. Tubular structures detected in the cortical region of the granule were more numerous than those noted in the disrupted eosinophil granule of man. The different reaction to injury of the eosinophils of man and those of the guinea pig appears to be characteristic for each of the two species. (Amer J Path 65:311–324, 1971)

CHARCOT-LEYDEN CRYSTALS are microscopic structures apparently formed by aggregation of material from disintegrating eosinophilic leukocytes. The crystals develop where localized concentrations of eosinophils occur. In man, Charcot-Leyden crystals are noted in the sputum of patients with bronchial asthma,[1,2] pulmonary ascariasis and tropical eosinophilia.[3] In feces, they are regularly seen in the dysenteric stools of patients with amebic, *Trichuris* or ulcerative colitis.[4] In tissues, Charcot-Leyden crystals are found in eosinophilic granulomas of bone [5] and in granulomas associated with tissue invading helminths, particularly visceral larva migrans.[6]

Charcot-Leyden crystals, which are easily recognized by light microscopy, are colorless and have the appearance of two hexagonal pyramids

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placed base to base, with opposing triangular faces forming an angle of 20° at the apex.[1] The usual crystal measures 20–40 μ in length and 2–4 μ at the broadest portion. Chemically, they are composed of a single polypeptide of low molecular weight [7] from which 14 different amino acids have been identified.[8] Although the structure and enzymatic content of the granules of eosinophilic leukocytes from several species are basically similar,[9–17] Charcot-Leyden crystals have been found only in man and monkey.[18] This curious absence of Charcot-Leyden crystals in the tissues of nonprimate animals prompted the present investigation.

Charcot-Leyden crystals can be produced *in vitro* from degenerating eosinophils.[19] The early intracellular stages of the crystal formation have been observed by electron microscopy.[20] In the present study, electron microscopic observations were made on Aerosol-treated (degenerating) and untreated human eosinophils to determine the intracellular changes leading to the formation of Charcot-Leyden crystals, and on guinea pig eosinophils subjected to the same treatment. Because a large sample of eosinophils was needed, it was impractical to use normal blood and the samples were obtained from experimental animals and from human beings with elevated levels of circulating eosinophils.

Materials and Methods

Human blood samples were obtained by venipuncture from 7 patients at the Lallie Kemp Charity Hospital of Louisiana. The patients had helminthic infections, including suspected cases of visceral larva migrans, toxocariasis or eosinophilia of undetermined etiology. The patients had levels of blood eosinophilia ranging from 15 to 60%.

In guinea pigs, eosinophilia was induced by repeated biweekly oral infection with 5000 infective larvae of *Toxocara canis,* which were obtained and cultured according to the method of Oshima.[21] Blood samples from 6 guinea pigs were taken by cardiac puncture when eosinophils reached levels of 15–20% of the circulating white blood cells. The following procedures were used for the human and the guinea pig blood. Sodium citrate (0.25 mg/ml of blood) was added to each blood sample to prevent clotting. Blood samples of 5 ml each were immediately centrifuged at 800 rpm for 15 minutes to aggregate all leukocytes in a buffy coat and to separate the plasma, which was removed. Aerosol-OT (anionic dioctyl sodium sulfosuccinate, American Cyanamid Co) in amounts of a few drops was then layered over the buffy coat. The blood samples were exposed to Aerosol OT for periods of 3–5 minutes, 1 hour, 24 hours or 72 hours. Samples not treated with Aerosol OT were used as controls. The treated cells were washed with fixative several times to remove residual Aerosol OT. The entire buffy coat from all samples was processed after the method of Anderson,[22] then embedded in Maraglas epoxy resin.[23] Sections of the Maraglas-embedded blocks were cut 1 μ thick with glass knives, stained with Paragon 1301 [24] and examined with the light microscope to identify and study the character of the eosinophils and Charcot-Leyden crystals if present. Thin sections were cut with glass knives on an LKB Ultratome III microtome, mounted unsupported on copper grids, stained and examined in a Siemens Elmiskop I or Philips EM-300 electron microscope.

For comparison with the blood samples, Charcot-Leyden crystals were obtained from stool samples from patients at Charity Hospital of Louisiana in New Orleans, by the methods described by Hornung.[7]

Results

Controls

The ultrastructure of the untreated control eosinophils from humans and guinea pigs was the same as that described by others for the normal eosinophil.[9,13,14,25] In both the human and guinea pig, the eosinophilic granule had a limiting membrane, a cortex with a granular matrix of moderate electron density and an electron-dense core. In thin sections, the granules were elliptic, oblong or nearly circular in outline; however, all of these shapes are consistent with a discoid-shaped granule. The core appeared as a thin rectangular bar when the granule was elliptic, a broad rectangular bar when the granule was oblong, or broadly rectangular, square or irregular in contour when the granule was circular in outline (Fig 1 and 3). It has been postulated that the core is plate-like and is situated in the equatorial or horizontal plane of the granule.[15]

Treated Human Blood

Charcot-Leyden crystals were detected by light microscopy in samples of buffy-coat leukocytes treated with Aerosol OT for 3–5 minutes or in those samples left untreated in the refrigerator for 72 hours. The crystals observed were hexagonal dipyramids and their description agreed in all respects with those of previous studies.[1,7,19]

By electron microscopy, cell lysis was observed in human eosinophils treated with Aerosol OT for 3–5 minutes. The plasmalemma was disrupted, numerous clear vesicles appeared in the cytoplasm, the endoplasmic reticulum was swollen, as were the mitochondria, and the mitochondrial cristae were disrupted. Often the inner and outer nuclear membranes were widely separated; in some cases the nuclear membrane was ruptured and the peripheral chromatin was absent.

Intracellular structures with a pattern resembling that of the relatively large (20–40 μ) Charcot-Leyden crystals were noted in many of these cells. These crystalline structures appeared identical to those described by Welsh.[20] He termed these ultracellular structures Charcot-Leyden crystals because of their morphologic resemblance to the larger extracellular crystals. His terminology appears valid and is used here. Charcot-Leyden crystals which were identifiable by their spindle shape or hexagonal cross section were present in the cytoplasm of the

eosinophil. In some cases, the crystals extended beyond the cell boundary.

In the samples treated with Aerosol OT, the limiting membrane of the granule of the eosinophil was thickened or replaced by fine granular material. The moderately electron-dense granular matrix of the cortex of the granule was absent. The core was no longer seen as a discrete structure and its contents appeared to be dispersed within the boundary of the granule. In the dispersed material of the core, there were granules of the same size and electron density as the material making up the Charcot-Leyden crystal. In some cases, the degenerative eosinophilic granules were seen in continuity and partially enclosed by the intracellular Charcot-Leyden crystals. This was interpreted to be a process of incorporation of the granular material into the Charcot-Leyden crystal (Fig 2). The granules of human eosinophils treated with Aerosol OT for 24 hours contained tubular structures 200–250 Å in diameter. These tubules were scattered within the cortex of the granule. The tubular structures resembled those found in the guinea pig cells and are described below.

Treated Guinea Pig Blood

In guinea pig eosinophils, Charcot-Leyden crystals were not detected by light microscopy even when Aerosol OT treatment was extended to 72 hours.

By electron microscopy, in guinea pig eosinophils treated with Aerosol OT for 3–5 minutes, no change could be detected, but at 1 hour the features associated with cell lysis, similar to those seen in human eosinophils treated for 3–5 minutes, were evident. Some eosinophilic granules remained unaffected, whereas others showed varying degrees of loss of the granular matrix. The core was usually intact, but there was disruption and focal loss of electron density of the core in some cases (Fig 4).

After 24 hours of Aerosol OT treatment, the cortical matrix was absent in all the granules (Fig 5). In the region of the cortex of the granule, where the matrix was orginally noted, there were numerous tubular structures 160–200 Å in diameter having an electron-dense periphery. In transverse sections of the granule, the tubules were clustered at the edge of the core. In sections cut parallel to the long axis of the granule, a dispersed granular mass having an electron density similar to that of the core occupied most of the granule. This dispersed granular mass had a pitted appearance, the diameter of the "pits"

being approximately equal to that of the tubular structures (Fig 6). No Charcot-Leyden crystals were detected.

Discussion

The formation of a Charcot-Leyden crystal is no less curious than that of the granule of the eosinophilic leukocyte. The function or functions of the granule of the eosinophilic leukocyte are incompletely understood. The cortical part of the granule has enzymatic activity, suggesting it functions as a lysosome.[11,26,27] The core of the granule apparently does not possess such enzymatic activity. Considering the complex structure of the eosinophilic granule, one wonders if the term lysosome adequately describes the entire granule.

In the present study, we demonstrated similarities of and differences between the altered eosinophil of man, where Charcot-Leyden crystals develop, and the altered eosinophil of guinea pig, where the crystals do not form. We can not advance a reason for the difference in behavior of the cells, but we can conclude that the guinea pig eosinophil is more resistant to damage by an injurious agent (in this case Aerosol OT) not only with respect to the morphologic integrity of the granule, but also with respect to the cell membrane and cell organelles. The guinea pig eosinophil will break up, but even when broken the Charcot-Leyden crystals do not form.

Various parts of the eosinophil have been considered as the site from which Charcot-Leyden crystals develop. Ayres [19] postulated that Charcot-Leyden crystals originate from the nuclei of degenerating eosinophils. We have rejected this hypothesis because we found the nuclei intact in most eosinophils where small intracellular Charcot-Leyden crystals were present. Archer and Blackwood [28] concluded that the crystals are derived from the soluble cytoplasmic portion of the eosinophil. Most workers have suggested the granule of the eosinophils as the source of the crystal. On the basis of electron microscopic studies, Welsh [20] concluded that the granules are the cellular structures most involved in the formation of Charcot-Leyden crystals. We agree with his conclusion and further add that the core of the granule may be the critical component.

The core of the intact eosinophilic granule of man is composed of small particles arranged in a lattice or crystalline array.[15] No repeating lattice has been convincingly demonstrated in Charcot-Leyden crystals, although a suggestion of such structure can be seen.

It is postulated that in the damaged eosinophil granule the crystalline configuration of the core becomes dispersed, then assumes the

configuration of the Charcot-Leyden crystal. A newly formed crystal probably serves as a nidus into which other disrupted granules become incorporated. There is no question that the Charcot-Leyden crystal seen by light microscopy is a crystal.

Eosinophils can phagocytize *Mycoplasma* organisms,[29] antigen-antibody complexes[30] and bacteria and zymosan particles.[31] Eosinophils degranulate during phagocytosis as do neutrophils.[32] However, the fate of the crystalline core after degranulation is apparently different in eosinophils of different species. In human eosinophils, the core could be detected within phagosomes 4 hours after incubation with *Mycoplasma* when the microorganisms were no longer recognizable.[29] In guinea pig eosinophils, the crystalline core was rarely encountered in granules that had fused with phagocytic vacuoles.[31]

In the present study, different patterns of reaction were observed in eosinophils of different species when they were subjected to an injurious agent. After treatment with Aerosol OT, almost all cell membranes and the membranes of cell organelles were disrupted, but a boundary (membrane-like structure) around the eosinophilic granule persisted. This boundary was more easily recognized in guinea pig than in human eosinophilic granules after equal periods of treatment. It seemed doubtful that such a boundary retained the functions of a limiting membrane. The cortex of the granule lost its granular matrix after treatment with Aerosol OT. Possibly, enzymes normally found in the cortex of the granule were in a soluble form and no structure could be seen or they escaped into the general cytoplasm. It was not within the scope of the present study to determine the histochemical localization of enzymes.

Histochemical findings[11,33,34] were interpreted as indicating that the lytic enzymes were lost after disruption of the eosinophilic granule. An insoluble protein fraction, which was assumed to be the core of the granule, was left behind. By electron microscopy, we found that the core and the tubular structures were the only components remaining in the granules of treated eosinophils. Progressive disruption of the structure of the eosinophilic granule was seen with loss of the granular matrix of the cortex, the appearance of tubular structures and dispersion of the core of the granule. These changes occur more slowly in the eosinophil of the guinea pig than in that of man.

The tubular structures that were noted in both human and guinea pig eosinophils after treatment with Aerosol OT for 24 hours and that were especially numerous in the guinea pig eosinophil, resemble microtubules in size and appearance. They may be an integral structural component whose presence can be detected only after the granular

matrix of the cortex of the eosinophilic granule is removed. Alternatively, they may represent new structures which arise as a consequence of Aerosol OT treatment.

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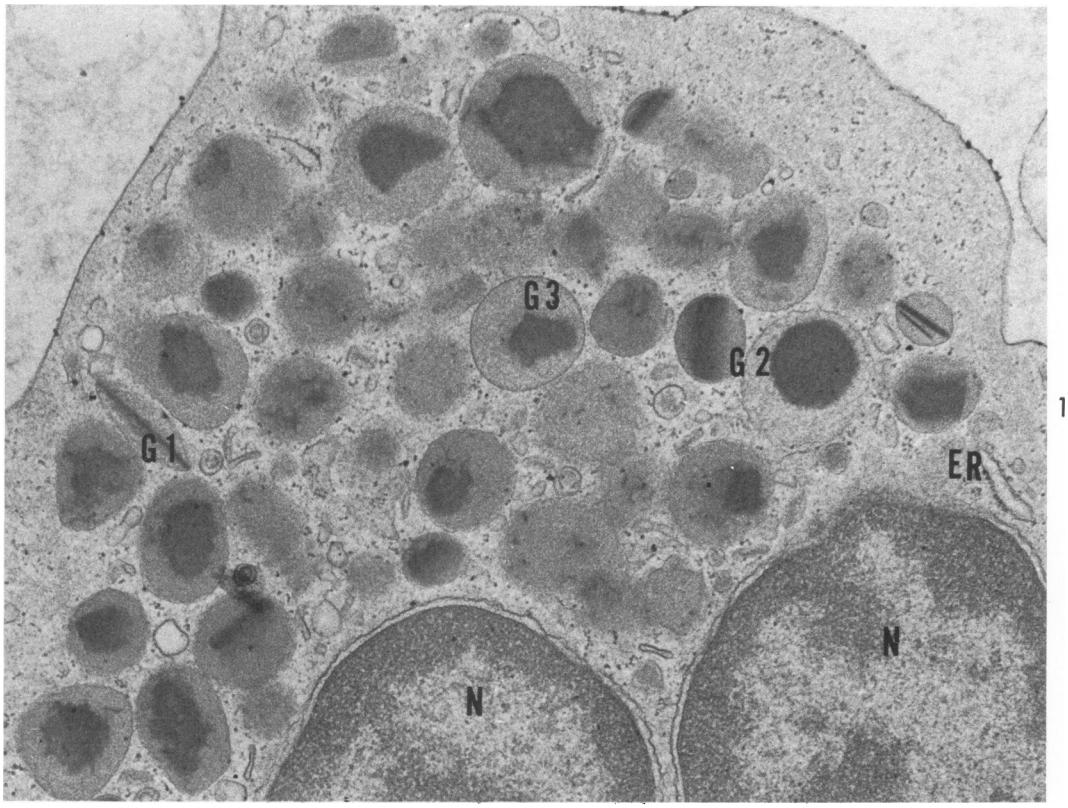
[*Illustrations follow*]

Fig 1—Untreated human eosinophil. In this electron micrograph, the eosinophilic granules and their cores display a variety of shapes: the core is a slender bar when the granule is elliptic (*G1*), a broad bar when the granule is oblong (*G2*), square or irregular when the granule is circular (*G3*). *ER* indicates endoplasmic reticulum; *N*, nuclear lobe (× 28,000).

Fig 2—Human eosinophil treated with Aerosol OT. The disrupted granules show loss of granularity in the cortex of the granule (*C*), accumulation of granular material at the periphery of the disrupted granule (*AG*) and dispersion of the core material and its fusion with the Charcot-Leyden crystal (*G*). *N* indicates nuclear lobe; *CLC*, Charcot-Leyden crystal (× 45,000).



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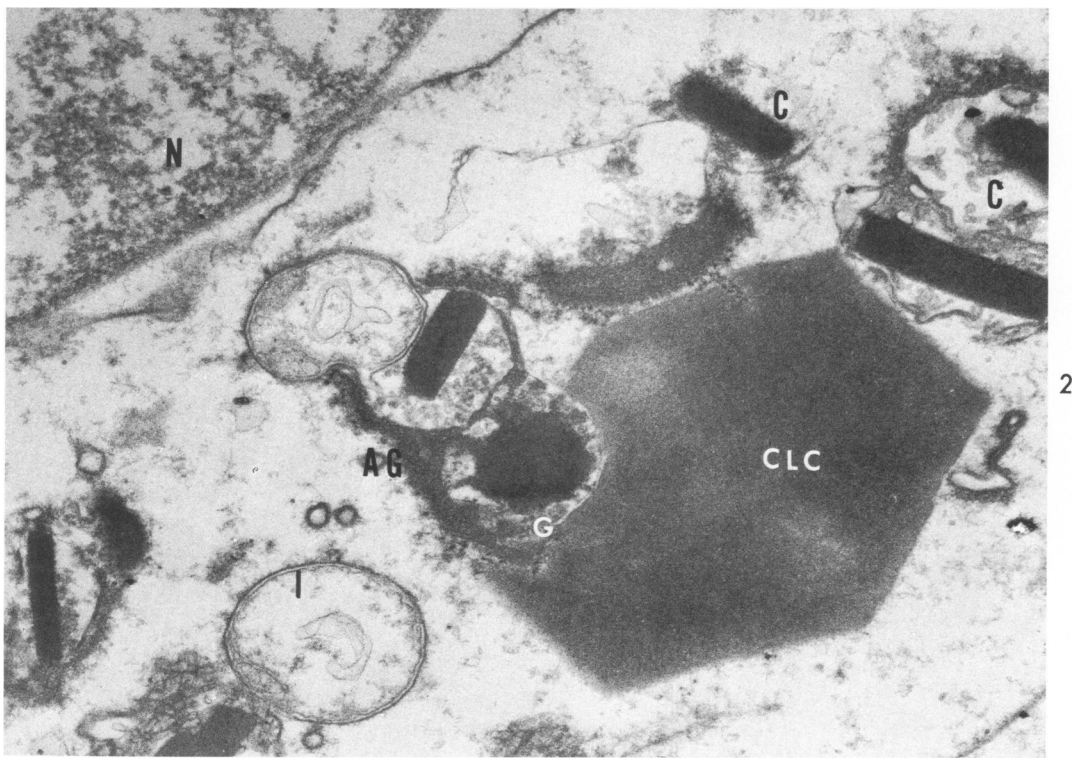

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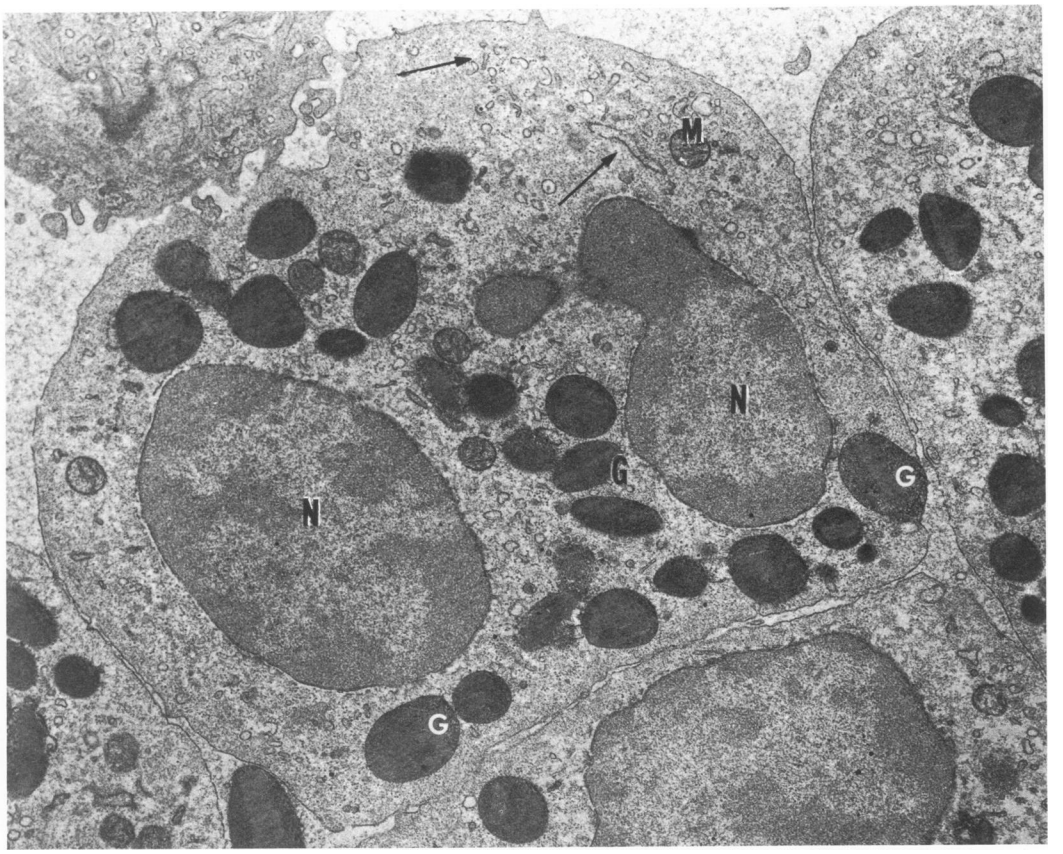
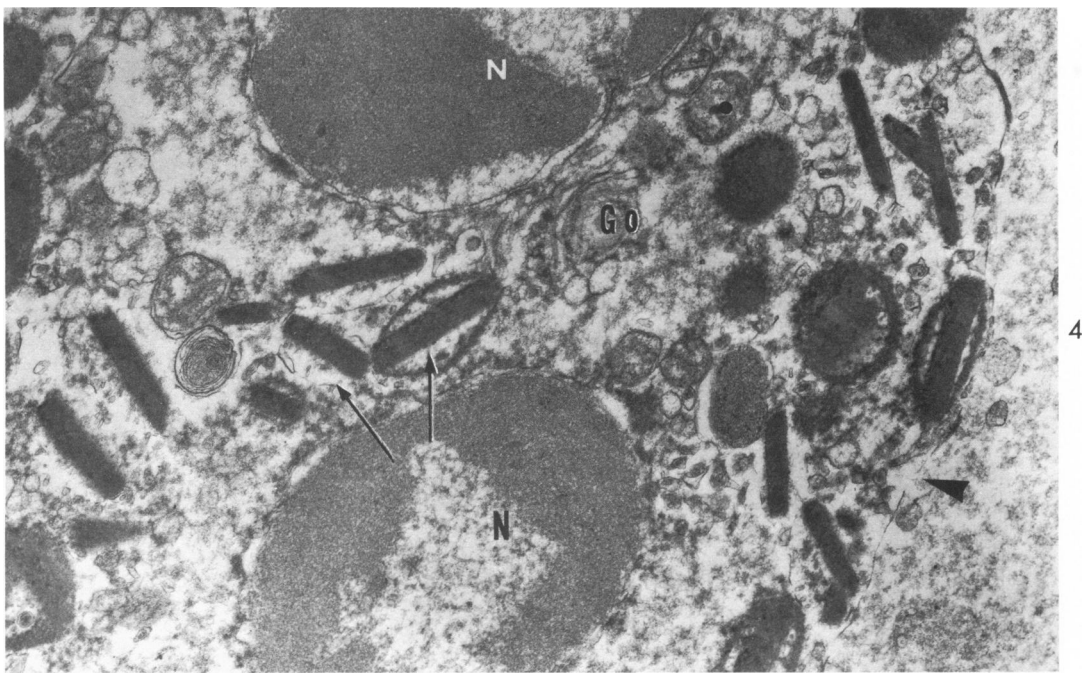


Fig 3—A normal eosinophil from the guinea pig. Eosinophlic granules (*G*) show the characteristic core and the granular cortex. In this picture, many of the cores are less electron dense than the cortical regions. *M*, indicates mitochondrion; *N*, nuclear lobe; *arrows*, endoplasmic reticulum (× 13,000).

Fig 4—Eosinophil from guinea pig blood after treatment for 1 hour with Aerosol OT. Eosinophilic granules show total or partial loss of the granular cortex (*arrows*). The plasmalemma is disrupted in some places (*arrowheads*). *Gc*, Golgi complex; *N*, nuclear lobe (× 21,000).

Fig 5—Eosinophil from guinea pig blood after treatment for 24 hours with Aerosol OT, showing extensive lysis of the cell. The plasmalemma is disrupted in several places (*arrowheads*). Eosinophilic granules (*arrows*) are shown in Fig 6. *N*, nuclear lobe (× 21,000).



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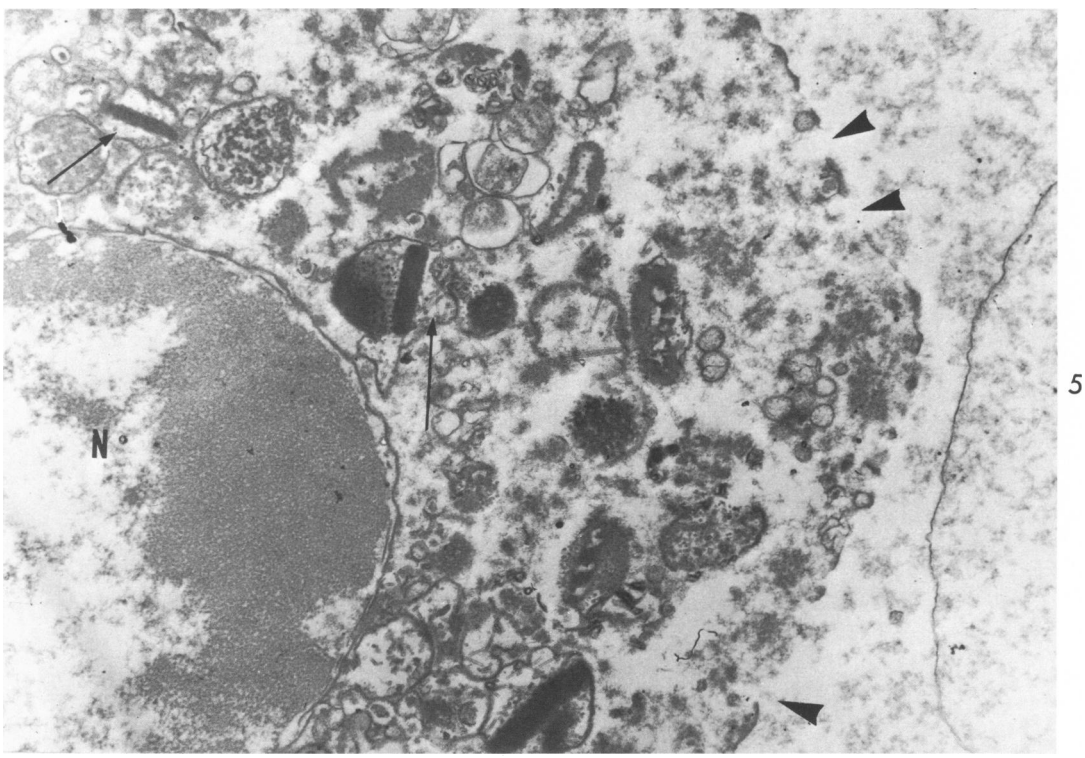

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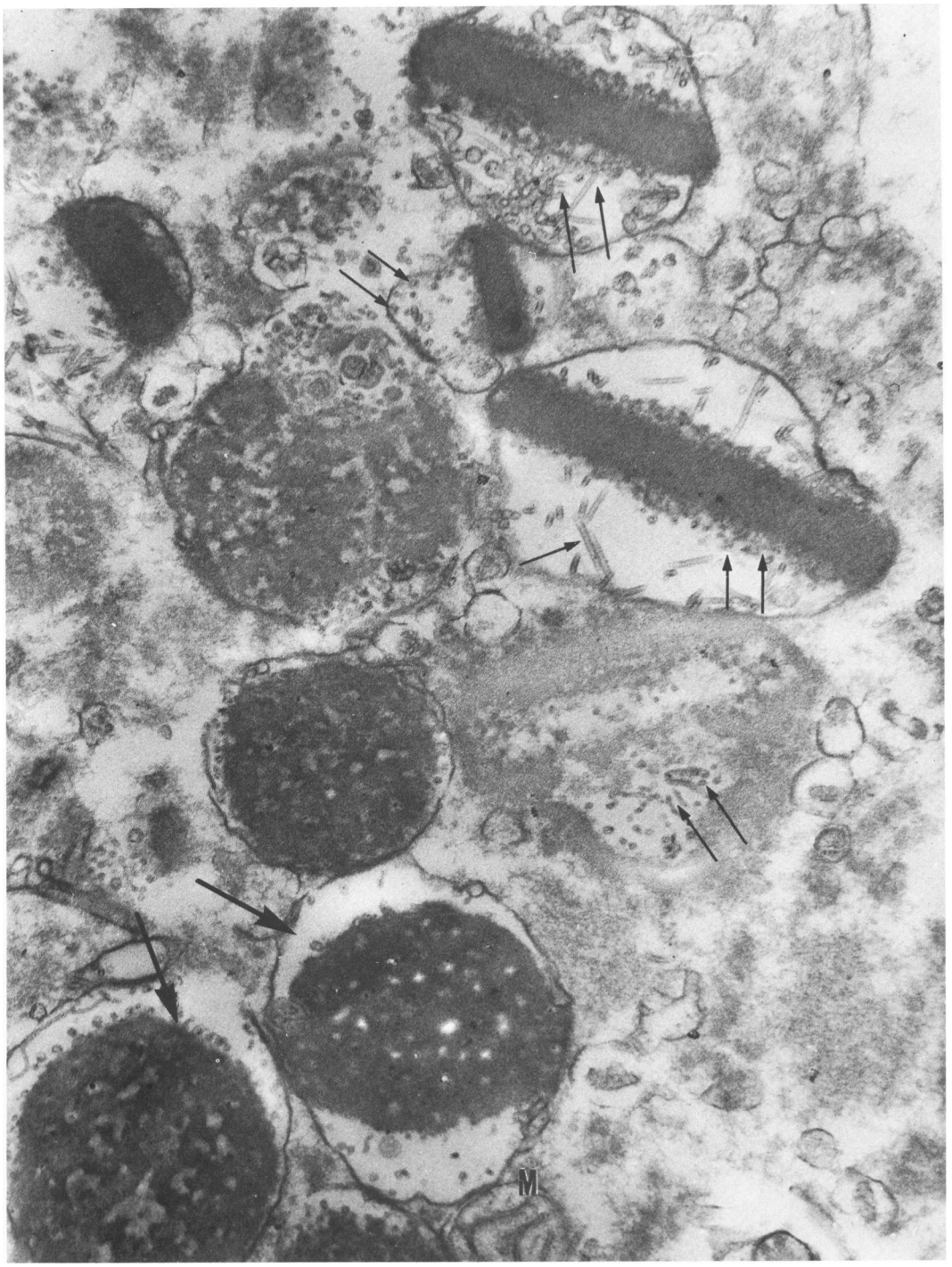

Fig 6—Part of an eosinophil from guinea pig blood after treatment for 24 hours. The absence of the granular matrix of the eosinophilic granule is evident. Tubular structures (*light arrows*) are detected in the cortex of the granule, particularly at the edge of the core. The irregular channeling and pitting of the core is evident when these are sectioned horizontally (*heavy arrows*). One mitochondrion (*M*) with intact cristae is present (× 52,500).